UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/580,987	05/25/2006	Zhiwen Zhang	54-001021US	5846

22798 7590 07/24/2009

QUINE INTELLECTUAL PROPERTY LAW GROUP, P.C.
P O BOX 458
ALAMEDA, CA 94501

EXAMINER	
LEAVITT, MARIA GOMEZ	
ART UNIT	PAPER NUMBER
1633	
MAIL DATE	DELIVERY MODE
07/24/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/580,987	ZHANG ET AL.	
	Examiner	Art Unit	
	MARIA LEAVITT	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 May 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *34-55 and 63-69* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *34-55 and 63-69* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 05-27-2009 has been entered.

1. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
2. Status of claims. Claims 34-55 and 63-69 are pending and not claims 34-45, 47 and 63-69 as applicants allege at page 1 of the remarks filed on 05-27-2009. Claims 34, 35, 44, 53 and 66 are currently amended and claims 68 and 69 have been added by Applicants' amendment filed on 05-27-2009.
3. Therefore, claims 34-55 and 63-69 are currently under examination to which the following grounds of rejection are applicable.

Response to arguments

Withdrawn Rejections/Objections in response to Applicants' arguments or amendments

Claim objection

In view of Applicants' amendment of claim 35 to delete the phrase "comprises a tryptophan analog", objection to claim 35 has been withdrawn.

Rejections/Objections maintained in response to Applicants' arguments or amendments

Claim Rejections - 35 U.S.C. § 112

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claim 41 remains rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 41, subpart c) recites "which hybridizes under highly stringent conditions over an entire length of a polynucleotide sequence of (a) or (b)". Highly stringent conditions of hybridization encompass different hybridization temperatures and salt concentrations in hybridization buffers. Therefore, the skilled artisan would not readily appraise the metes and bounds of "highly stringent conditions" nor how to assess such. It is unclear how to interpret what is considered "highly stringent conditions ". Thus the meaning and the metes and bounds of the claim as whole are unclear.

Claim Rejections - 35 USC § 112-first paragraph-

Please, note that the scope of enablement has been broaden in view of Applicants' remarks, in light of the guidance provided in the specification and knowledge available to one of ordinary skill in the art at the time of filing the present application and further in view of reconsideration of search under different premises

Claims 34-55, 63-67 remain rejected and new claims 68-69 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

A method of incorporating a 5-substituted tryptophan unnatural amino acid into a peptide, the method comprising,

(i) preparing a construct comprising a nucleic acid sequencing encoding an orthogonal mutant tryptophanyl-tRNA synthetase (O-muTrpRS) comprising at least 90% identity to the amino acid sequence of SEQ ID No. 2, the O-muTrpRS comprising a proline residue at position corresponding to position 144 of SEQ ID NO: 2, wherein the O-muTrpRS aminoacylates an orthogonal tRNA (O-tRNA) of SEQ ID No: 3 with the 5-substituted tryptophan, a 5-substituted tryptophan analog or a 5-hydroxy-L-tryptophan (5-HTTP) when the O-tRNA, 5-substituted tryptophan or analog thereof, 5-HTTP and the O-muTrpRS are present in an eukaryotic cell,

(ii) preparing a construct comprising a nucleic acid sequencing encoding an O-tRNA comprising at least 90% identity to the amino acid sequence of SEQ ID No: 3, wherein the O-tRNA is aminoacylated with the 5-substituted tryptophan analog or 5-HTTP by the O-muTrpRS of SEQ ID No. 2, when the muTrpRS, 5-substituted tryptophan analog or 5-HTTP and the O-tRNA are present in an eukaryotic cell,

(iii) introducing the O-muTrpRS construct and the O-tRNA construct into the eukaryotic cell, and

(iv) preferentially aminoacylating an expressed O-tRNA with the unnatural amino acid, wherein said aminoacylation is catalyzed by an expressed O-muTrpRS, whereby the 5-substituted tryptophan unnatural amino acid is incorporated into the peptide cell,

does not reasonably provide enablement for a genus of unspecified O- that aminoacylates a reference tRNA of SEQ ID NO:2 RS (e.g., the O-muTrpRS polynucleotide sequence of SEQ ID NO: 1 encodes the amino acid sequence of SEQ ID NO: 2) and a genus of undetermined O-tRNA that are aminoacylated by a reference O-RS.

The claims when given the broadest reasonable interpretation encompass a genus of nucleic acid sequences encoding an orthogonal aminoacyl-tRNA synthetase comprising at least 90% identity to SEQ ID No. 2, the O-muTrpRS comprising a proline residue at position

corresponding to position 144 of SEQ ID NO: 2, wherein a O-RS aminoacylates an orthogonal tRNA (O-tRNA) of SEQ ID No: 3 with a 5-substituted tryptophan unnatural amino. The claimed O-RS can be broadly interpreted as comprising any prokaryotic non mutated O-RS that does not require having at least 90% identity to SEQ ID No. 2, and functionally able to aminoacylate an orthogonal tRNA (O-tRNA) of SEQ ID No: 3 with a 5-substituted tryptophan unnatural amino. However, because there is not structure/function relationship taught at all for the genus of O-RS from prokaryote other than the structure/function relationship taught for a mutated tryptophanyl-tRNA synthetase of SEQ ID NO:2 or sequences at least 90% identical to SEQ ID NO:2, the claimed O-RS may not retain full or even partial functional activity to aminoacylate an orthogonal tRNA (O-tRNA) of SEQ ID No: 3 with a 5-substituted tryptophan unnatural amino. In other words, the O-tRNA may not be acylated at all by the genus of O-RS, thereby being unable to insert a 5-substituted tryptophan unnatural amino into the peptide in the eukaryotic host cell. Note that the instant claims are reading eukaryotic cell so the O-RS has to be catalytically competent in said eukaryotic cell.

Additionally, claim 41 subparts (a) and (b) are drawn to an O-muTrpRS construct comprising a nucleic acid consisting of SEQ ID NO: 1 and a conservative variant thereof, and to a nucleic acid encoding a polypeptide consisting of SEQ ID NO:2 and a conservative variant thereof. The specification as filed defines "Conservative variations" at paragraphs [0092] to [0094] as nucleic acids which encode identical or essentially identical amino acid sequences (see, Table 1). Hence the claimed nucleic acid variants can be modified at any position of the nucleotide sequence of SEQ ID No. 1, provided that they encode conservative amino acid residues. Thus the breadth of claim 41 remains very broad. While one of skill in the art can

readily envision numerable species of nucleic acid sequences that are at least a given % identity to a reference nucleotide sequence (e.g., SEQ ID No. 1), that encodes a variant of at least a given % identity to the amino acid sequence of SEQ ID No. 2, one cannot envision which of these also encode a *B. subtilis* O-muTrpRS with the claimed orthogonal activity (e.g., one catalytically competent in an eukaryotic cell). Moreover, claim 41 subparts (c), (d) and (e) read on nucleic acid variants and/or fragments of the SEQ ID NO: 1 gene encoding amino acid SEQ ID No. 2 comprising changes, deletion, or addition, may alter the conformation/structure of the O-muTrpRS in unknown ways. Furthermore, in terms of the structural requirements of the O-mTrpRS nucleic acid molecules, claim 41 subparts a)-d) recites an arbitrary structural relationship between the claimed nucleic acid sequence(s) and the single disclosed species of nucleotide sequence and amino acid sequence, respectively, based upon hybridization of nucleic acid. Hybridization of two nucleic acids, even under high stringency conditions, requires only that the two nucleic acids share between 25 and 50 nucleotides in common. (Kennell, Progr Nucleic Acid Res. Mol. Biol. 11: 259-301, 1971, at the paragraph bridging pages 260-261). Such a sequence encodes only 8-16 amino acids. Consequently the claims embrace polypeptides that could share as few as 8-16 contiguous amino acids in common out of the 330 amino acids of SEQ ID NO: 2. Conversely, a nucleotide sequence that differs in every wobble base from SEQ ID NO: 1 of 993 nucleotides, for example, would encode SEQ ID NO: 2, but would not detectably hybridize to SEQ ID NO: 1 under any conditions. Thus, the recited structural relationship is arbitrary since neither the specification nor the prior art discloses any definitive relationship between protein function and % identity or homology at the nucleotide level; and the specification does not describe a single species of nucleic acid that encodes a functional protein

that is not at least 90% identical to SEQ ID NO: 1 or that encodes a polypeptide that is not at least 90% identical to SEQ ID NO: 2. Thus, claim 41 reads on structural features, i.e., percent identity, percent hybridization, which specifies a family of unrelated structurally- and functional - O-muTrpRS -that need not to retain full or even partial activity.

The specification provides sufficient guidance in Example 1 for the corresponding pair O-TrpRS/ O-tRNA from *B. subtilis* that can be used to genetically encode an unnatural amino acid (and not endogenous amino acids) in mammalian cells, because the *B. subtilis* O-tRNA is not recognized by any of the aminoacyl-tRNA synthetases in the mammalian endogenous translation system thus preventing aminoacylation of the O-tRNA with endogenous amino acids. Hence Example 1 confirms the inter-species differences in tRNA recognition elements. In other words, *B. subtilis* O-TrpRS can efficiently charge total tRNA isolated from *B. subtilis* including a *B. subtilis* tRNA with mutations of the anticodon loop *(i.e., B. subtilis* $tRNA^{Trp}$). Additionally, paragraph 170 describes the generation of the O-RS/O-tRNA orthogonal pairs taking advantage of inter-species differences in tRNA recognition elements, particularly the knowledge that *B. subtilis* $tRNA^{Trp}$ is generally not a substrate for the tryptophan-tRNA synthetases from yeast and mammalian cells, clearly indicating that *B. subtilis* $tRNA^{Trp}$ was the starting point of the invention as it appears to be orthogonal to mammalian cells. Furthermore, Example 2 teaches the uniqueness of orthogonal pair *B. subtilis* orthogonal tryptophanyl-tRNA synthetase (O-muTrpRS)-opal suppressor tRNA ($^{mut}tRNA^{Trp}_{UCA}$) after transfecting mammalian 293T cells with three individual plasmids, pTrptRNA, pFoldon TGA (e.g., UGA termination mRNA selector codon) and mutant pEF6-TrpRS (i.e.,Val144ProBsTrpRS), demonstrating that opal suppression (UCA) in mammalian cells depends on expression of the *B. subtilis* orthogonal tryptophanyl-

tRNA synthetase (O-muTrpRS)/opal suppressor tRNA ($mutRNA^{Trp}_{UCA}$) pair. Thus, *B. subtilis* orthogonal tryptophanyl-tRNA synthetase (O-muTrpRS) aminoacylates the corresponding *B. subtilis* opal suppressor tRNA ($mutRNA^{Trp}_{UCA}$) with 5-HTPP for suppression of the TGA68 in the mutant foldon construct. In other words, the generation of the *B. subtilis* tryptophanyl-tRNA synthetase (O-TrpRS)-opal suppressor $tRNA^{Trp}$($mutRNA^{Trp}_{UCA}$) pair uniquely incorporates 5-HTTP (e.g., an unnatural amino acid) into a mammalian protein in response to a UGA termination anticodon and the mRNA selector codon. Thus, the orthogonal *B. subtilis* is pair specific and replacement of one element of the pair such as the *B. subtilis* O-tRNA by other species O-tRNA, e.g., *B. stearothermophilus* t-RNA^{Trp} or *E. coli* t-RNA^{Trp}, would necessitate a different cognate TrpRS to enable the claimed functionality. Paragraph 140 refers to well known elements in the tRNA sequences of prokaryotes and eukaryotes that influence expression activity and specifity of tRNA enzymes. The disclosure does not provide sufficient guidance for the structure/function relationship of twenty one cognate amynoacyl-tRNA synthetase- tRNA pairs to be used in site-specific incorporation of amino acid analogs into proteins in prokaryotes and eukaryotes. Clearly, apart from the *B. Subtilis* orthogonal tryptophanyl-tRNA synthetase (O-TrpRS)-opal suppressor $tRNA^{Trp}$ ($mutRNA^{Trp}_{UCA}$) pair, the as-filed specification does not provide sufficient disclosure for selection and use of other conservative variants of amino acid sequences that are not at least 90% identical to SEQ ID NO: 2 (O-muTrpRS) and a genus of unspecified species of O-tRNAs that are not at least 90% identical to SEQ ID NO:3 able to selectively incorporate a 5-substituted tryptophan unnatural amino acid into proteins of eukaryotic cell. There is no description in the specification, as originally filed, of other mutations of other claimed genus of O-tRNA/O-RS pairs wherein the O-RS uniquely recognizes the O-

tRNA and selectively charges it with a 5-substituted tryptophan unnatural amino acid, as embraced by the claim limitations. Hence, there is not indication of a structure-function relationship between a genus of O-RS nucleic acid sequences encoding conservative variants SEQ ID NO: 2 and the claimed genus of species of the O-tRNA, other than $tRNA^{Trp}$($mutRNA^{Trp}_{UCA}$) or amino acid sequences at least 90% identical to SEQ ID NO: 3 for the preferential aminoacylation of the O-$tRNA^{Trp}$ with a 5-substituted tryptophan unnatural amino acid so as for the acylated RNA to insert the 5-substituted tryptophan unnatural amino. No disclosure of other *B. subtilis* tRNA cross-species tRNA anticodon specifity or tRNA cross-species tRNA specifities of synthetases are disclosed, let alone any tRNA (other than *B. subtilis* tRNA) cross-species tRNA specifities of synthetases for site-specific incorporation of 5-subtituted tryptophan unnatural amino acid. There is not evidence that any nucleic acid sequence encoding an orthogonal conservative variant of SEQ ID NO: 2 (O-muTrpRS) would efficiently charge an O-tRNA from any species with a 5 substituted tryptophan unnatural amino acid into a peptide in an eukaryotic host cell. In other words, there is not evidence that any O-RS can properly interact with a tRNA of SEQ ID NO:3 other than an O-muTrpRS comprising at least 90% homology to SEQ ID NO: 2. The disclosure merely amounts to an invitation for the skilled Artisan to try and follow the disclosed instructions of known motifs of tRNA synthetase and tRNA to make and use the claimed orthogonal pair of the invention. How can such a broadly claimed evaluation step be performed without an undue experimentation when there is no supporting evidence to substantiate a reasonable correlation of how any of the amynoacyl-tRNA synthetase aminoacylates the corresponding suppressor tRNA and no other endogenous tRNA in the cell other than the amynoacyl-tRNA synthetase comprising at least 90% homology to the amino acid

sequence of SEQ ID NO: 2, or how a suppressor tRNA is not aminoacylated by any of the endogenous amynoacyl-tRNA synthetase in a method that specifically incorporates a 5-subtituted tryptophan unnatural amino acid in an eukaryotic host system other than the O-tRNA comprising at least 90% identity to the amino acid sequence of SEQ ID No: 3?

Response to Applicants' Arguments as they apply to rejection of Claims 34-55 and 62-69 under 35 U.S.C. 112, first paragraph.

At pages 9-10 of the remarks filed on 05-27-2009, Applicants essentially argue that, 1) the fact that not sufficient guidance is provided for the twenty one cognate amynoacyl-tRNA synthetase-suppressor tRNA pairs to be used in site-specific incorporation of amino acid analogs into proteins in prokaryotes and eukaryotes does not alter the fact that it would be reasonably easy for one of ordinary skill in the art to modify arms of the tRNA molecule with complimentary residue pairs without destroying the specific activity, 2) Because of the high general knowledge of RS and tRNA structure and function, the quantity of experimentation require to identify any number of modifications outside the required structures should be expected to retain activity, 3) the claims are not directed to the universe of any host system (e.g., prokaryote or eukaryote) but are directed to specifically taught functional eukaryotic systems; as the O-RS/tRNA pairs are specifically structured to functionally interact in a eukaryotic system (e.g., with the tRNA pseudo A box, and 5' flanking sequences - see paragraphs 140 and 172) to incorporate 5-substituted tryptophans (e.g., with specific mutations such as Pro144 - see paragraph 179) one of skill in the art, understanding the general structure of a RS/tRNA pair, with guidance of the many structures identified as correlating with functional activity, would be able to incorporate a 5-substiture tryptophan in an eukaryote without undue experimentation, and

5) Deiter et al (2003; JACS pp. 11782-11783) evidences how the orthogonal TyrRS/$tRNA_{CUA}$ pair in yeast which encode acetylene and azido amino acids has been used for incorporation of azide-or acetylene-containing unnatural amino acids into proteins in response to the amber nonsense codon, TAG. Applicants appear to infer that the instant claimed pair is analogous and would be reasonably expected to function in a similar fashion. The above arguments have been fully considered but deemed unpersuasive.

Regarding 1), 2) and 3), It is well established in the art that when the structure/functional correlation in a protein is well known, the modification of residues that generate a functional protein does not required undue experimentation. This is the case here. Modification of known residues in the arm structure of *B. subtilis* tryptophan opal suppressor –tRNA to generate a functional *B. subtilis* O-tRNA will require undue experimentation. However, the present issue of enablement is whether the scope of the patent protection sought by the Applicant as defined by the currently amended claims correlates with the scope of enabling disclosure set forth in the specification. In other words, is there enough disclosure for a generic pair O-RS and O- tRNA that is less than 90% homologous to the O-muTrpRS of SEQ ID NO:2 and less than 90 % homologous to the O- tRNA of SEQ ID NO: 3, respectively, wherein the O-RS specifically aminoacylates the O-tRNA with the 5-subtituted tryptophan unnatural amino acid? For the reasons already of record as set forth at pages 3-11 of the previous office action of 01-22-2009, said reasons in part reiterated in the paragraphs above, orthogonal O-RS/O-tRNA pairs exhibit inter-species differences in tRNA recognition elements. Additionally, the O-RS/O-tRNA is pair specific. For example, the orthogonal *B. subtilis* is pair specific and replacement of one element of the pair such as the *B. subtilis* O-$tRNA^{Trp}$ by other species O-tRNA, e.g., *B.*

stearothermophilus t-RNA^{Trp} or *E. coli* t-RNA^{Trp}, would necessitate a different cognate TrpRS to enable the claimed functionality. As such only those O-RS and O-tRNA sharing at least 90% homology with muTrpRS of SEQ ID NO: 2 and at least 90% identity with O-tRNA of SEQ ID NO: 3 could be generated and screened without undue experimentation. Regarding the flexibility in percent identity, it is well established in the art that there is a definitive relationship between protein function and % identity at either the amino acid or nucleotide level. Indeed, percent identity is highly predictive of protein function and without this tool it would be impossible to make meaningful annotations of genomes in sequencing projects. Proteins that share 90% amino acid identity are known to possess the same catalytic/biochemical function which has formed the basis for genome annotation and comparative genomics. In fact, 90% identity is a conservative criterion for judging functional similarity. A long history of structure-function studies has demonstrated that single domain proteins that share substantial similarity (and >30% identity) over their entire length (>80 residues) without introduction of numerous gaps are almost certainly homologous (derive from a common evolutionary ancestor) and share the same three-dimensional structure (see Marti-Renom et al., MA, Stuart AC, Fiser A, Sanchez R, Melo F, Sali A. Comparative protein structure modeling of genes and genomes. *Annu Rev Biophys Biomol Struct* 2000; 29:291-325). At the 90% level of amino acid identity, orthologous enzymes in related species are virtually guaranteed to share the same catalytic function and substrate specificity. Likewise, genes that share 90% identity encode proteins with the same catalytic/biochemical function. Indeed, the specification clearly discloses knowledge of the active site in the *B. subtilis* tryptopanyl-tRNA synthetase (BsTrpRS) determined by comparison to the structure of highly homologous nucleotide sequence of a *Bacillus stearothermophilus*

tryptophanyl-tRNA synthetase (paragraphs [0178]-[0179]). However, Applicants have not provided any evidence for structure/functional relationship of cognate amynoacyl-tRNA synthetase-suppressor tRNA pairs sharing less than 90% homology with muTrpRS of SEQ ID NO: 2 and less than 90% homology to O-tRNA of SEQ ID NO: 3 that could be generated and screened without undue experimentation to specifically incorporates a 5-subtituted tryptophan unnatural amino acid in eukaryotic host cells.

Regarding 4), the orthogonal TyrRS/tRNACUA pair in yeast which encode acetylene and azido amino acids which is the *E.coli* tyrosyl tRNA-tRNA synthetase pair, wherein the amino acid specifity of the *E.coli* tyrosyl -tRNA synthetase was altered to incorporate azide-or acetylene-containing unnatural amino acids into proteins in response to the amber nonsense codon, TAG is not dispute. However, Applicants have not provided any evidence that *E. coli* tyrosyl tRNA/tRNA synthetase pair is functionally homologous to the claimed *B. subtilis* O-RS/O-tRNA of the invention. Is the comparative alignment of the *E. coli* tyrosyl tRNA synthetase predictive of the structure and functionality of the *B. subtilis* O-RS or any generic O-RS? Because no structural features have been disclosed for the claimed O-RS comprising less than 90% identity to sequence ID NO:2 and O-tRNA comprising less than 90% identity to sequence ID NO:3 providing a correlation between function and structure, the disclosure is not deemed sufficient to enable the skilled artisan to use the claimed invention at the time the application was filed without undue and/or unreasonable experimentation.

New grounds of objection/ rejection

Claim Objection

Claims 41 and 42 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Clam 41 depends from claim 34, which is directed to nucleic acid molecules that encode a protein having at least 90% identity to SEQ ID NO: 2. In addition to being directed to a subset of such nucleic acid molecules, i.e. those comprising SEQ ID NO: 1 and conservative variations or to the polynucleotide sequence encoding SEQ ID NO: 2 or conservative substitutions, claim 41 also embraces fragments of SEQ ID NO:1, which would not encode a protein as required by claim 34. Note, for example, that any stretch of 100 or more consecutive homologous nucleotides to the nucleotide sequence of SEQ ID NO: 1 of 993 nucleotides can hybridize under high stringency, and as such these fragment are broadly encompassed by claim 41 subparts a) to d). Furthermore, claim 41 does not require the O-muTrpRS of SEQ ID NO:2 to properly interact with the corresponding O-tRNA. Thus, the scope of claim 41 extends beyond that of claim 34 from which it depends.

Likewise, claim 42 depends from claim 34, which is directed to nucleic acid molecules that encode a protein having at least 90% identity to SEQ ID NO:2. Claim 42 merely requires that the O-muTrpRS construct to comprise a mutated O-muTrpRS sequence of at least at one amino acid residue. Thus any O-muTrpRS sequence with one amino acid mutation would anticipate claim 42 but not claim 34, as the parent claim requires the O-muTrpRS to comprise at least 90% identity to SEQ ID NO:2. Furthermore, claim 41 does not require the O-muTrpRS of SEQ ID NO:2 to properly interact with the corresponding O-tRNA. Thus, the scope of claim 42 extends beyond that of claim 34 from which it depends.

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claims 47 and 50 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite in that it fails to point out what is included or excluded by the claim language

Claim 47 which depend from claim 34, is indefinite in the reciting "further comprising mutating a prokaryotic tRNA sequence". Claim 47 requires an O-tRNA comprising 90% homology to the SEQ ID NO: 3. Hence, it is unclear whether there are more than one orthogonal tRNA in the eukaryotic host cell or there are additional tRNA constructs encoding non orthogonal tRNAs. Therefore, the metes and bounds of "further comprising mutating a prokaryotic tRNA sequence" are not clearly set forth.

Claim 50 which depend from claim 34, is indefinite in the reciting "the O-muTrpRS construct and the O-tRNA construct comprise the same construct". The constructs of claim 34 comprise nucleic acid encoding the O-muTrpRS and O-tRNA. Thus it is unclear how these two proteins are comprised in the same construct that comprises a nucleic acid sequence. The meaning and the metes and bounds of the claim as whole are unclear.

Claim Rejections - 35 USC § 112- First paragraph- New Matter

> The following is a quotation of the first paragraph of 35 U.S.C. 112:
>
> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 34-55 and 63-69 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claim 34 has been amended to recite "or HTTP is incorporated". The response dated 05-27-2009 indicates where support for the amendments regarding aspect of binding and aminoacylation of tRNA of SEQ ID NO: 3 by O-RSs; or aminoacylation of O-tRNAs by RSs of SEQ ID NO: 2 can be found, e.g., at paragraphs 38, 70, 110, 130, 131,142 and 179; the section starting at paragraph 133, the Examples, and in Figures 1 and 5. There are no indications where support may be found for the above limitations regarding incorporation of HTTP, however. A review of the specification as filed reveals no specific disclosure of hydroxy-L-tryptophan (HTTP). What the specification does disclose regarding HTTP is that site-directed orthogonal mutagenesis was then used to alter the specificity of BsTrpRS to uniquely charge 5-hydroxy-L-tryptophan (5-HTTP) (p. 59, paragraph [0157]). There is nothing more to lead one of skill in the art to appreciate that any HTTP was part of the invention as opposed to 5-HTTP. Applicants have not provided any evidence that there are in possesion of a genus of unspecified hydroxy-L-tryptophan, let alone that L-tryptophan can be hydroxylated at any location other than 5' . Thus the specific embodiments regarding the breadth of HTTP sets forth a new range not previously disclosed as a contemplated embodiment in the present specification, nor one that was readily known and used in the art at the time of filing. Hence, is not clear that the Applicant was in possession of a genus of undefined "or HTTP is incorporated" at the time of filing.

Claims 34-55 and 63-69 will remains rejected until Applicant cancels all new matter.

Conclusion

Claims 34-55 and 63-69 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

/Maria Leavitt/

Maria Leavitt, PhD
Examiner, Art Unit 1633